Appl. No. : 08/779,457
Filed : January 7, 1997

AMENDMENTS TO THE SPECIFICATION

Please insert the following paragraphs at page 69, line 7, immediately prior to the paragraph beginning "The antibody of interest herein..."

For *ad libitum* feeding studies, 7-week-old genetically obese C57BL/6J-*ob/ob (ob/ob)* and C57BL/KsJ-*db/db (db/db)* mice and lean littermates (heterozygous C57BL/6J-+/*ob for ob/ob* and wild-type C57BL/KsJ-+/m for *db/db)* were purchased from The Jackson Laboratory. Mice were housed in groups of four or five with *ad libitum* access to water and standard mouse chow (Purina 5010) in a temperature-, humidity-, and light-controlled (lights on at 06:00 hr, off at 18:00 hr) colony room. Miniosmotic pumps (Alzet model 2002; Alza) were filled with purified recombinant ob protein (100 μg/kg per day) in sterile phosphate-buffered saline (PBS) or PBS alone under sterile conditions following manufacturer's instructions and incubated overnight in sterile saline at room temperature before implantation into mice. Mice were anesthetized with ketamine/xylazine, and miniosmotic pumps were implanted s.c. in the midscapular region.

The body weight of each mouse (to the nearest 0.1 g) and the weight of the food contained in the food bin in each case (to the nearest 0.1 g) were recorded between 17:00 hr and 18:00 hr every 1 to 2 days. Mice were killed by barbiturate overdoes followed by exsanguination via cardiac puncture. Fat pads and organs were immediately dissected, blotted, and weighed to the nearest 0.001 g. Hepatic glycogen content was assessed on paraffin-embedded liver sections that were fixed in 10% neutral-buffered formalin and stained by the periodic acid Schiff reaction with or without previous diastase digestion. Hepatic lipid content was assessed on fresh frozen liver sections that were stained by Oil Red O. Fat pads were histologically examined after fixation in 10% neutral-buffered formalin, sectioning, and hematoxylin/eosin staining. Blood samples (≈0.2 ml) were obtained from the retroorbital sinus of conscious mice on day 13 of treatment at

Appl. No. : 08/779,457
Filed : January 7, 1997

14:00 hr, after a 5-hr fast. Blood was stored on ice until centrifugation, and then serum was stored at -20°C until use. Serum insulin concentrations were determined by radioimmunoassay (Linco Research, St. Louis). Serum concentrations of glucose, cholesterol, and triglycerides were determined on a Technicon Chem1 + System chemistry analyzer (Bayer, Tarrytown, NY).

For pair-feeding studies, 8-week-old obese (C57BL/6J-*ob/ob*; The Jackson Laboratory) or lean (C57BL/6; Charles River Breeding Laboratories) female mice were housed as described above. The three treatment groups for each genotype were *ad libitum*-fed PBS-treated, *ad libitum*-fed ob protein-treated, or pair-fed PBS-treated. The ob protein was delivered via miniosmotic pumps as described above at a does of 270 μg/kg per day. Pair feeding was accomplished by measuring the food intake of the *ad libitum*-fed ob protein-treated mice every 24 hr and presenting this amount of food to the pair-fed PBS-treated mice. For each of the three treatment groups there were two to three cages of mice, containing two to five mice per cage. Blood samples were obtained, the mice were killed, and tissues were harvested as described above.

All data are presented as the mean ± SEM and were analyzed by ANOVA with *post hoc* differences determined by Fisher's protected least significant different test if ANOVA was significant at the level of $P < 0.05$.